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Knowledge of the neuronal membrane properties and synaptic physiology of the suprachiasmatic nucleus (SCN) is critical for an understanding of the cellular basis of circadian rhythms in mammals. The hypothalamic slice preparation from rodents and a combination of electrophysiological techniques (i.e., extracellular single- and multiple-unit recording, intracellular recording, and whole-cell patch clamp) were used to study (1) the role of excitatory and inhibitory amino acids (i.e., glutamate and GABA) in synaptic transmission, (2) the membrane properties of SCN neurons, and (3) the mechanisms of neuronal synchronization. Antagonists for N-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors blocked excitatory postsynaptic potentials (EPSPs) evoked by stimulation of the optic nerve other sites when SCN cells were depolarized or at rest, respectively. Bicuculline blocked inhibitory postsynaptic potentials (IPSPs) that were evoked by local stimulation or that occurred spontaneously. The IPSP reversal potential was near the Cl^- equilibrium potential, and was shifted to depolarized levels by raising intracellular [Cl^-]. Thus, glutamate and GABA appear to mediate fast excitatory inhibitory synaptic transmission in the SCN. Some SCN neurons, but not all of them, has low-threshold Ca^{2+} spikes and time-dependent inward rectification, thus indicating that the electrical properties of SCN neurons are not homogenous. Neurons with a firing rate >6 Hz had a regular pattern, and neurons with a rate <4 Hz had an irregular pattern; since both the firing rate and pattern could be modified with injected currents, SCN neurons with different firing patterns are unlikely to represent distinct classes of cells. Synchronous bursts of action potentials occurred in the SCN after chemical synapses were blocked with [Ca^{2+}]-free solutions and with amino-acid-transmitter antagonists, which indicates that synchronous neuronal activity can occur in the SCN without chemical synapses and suggests that different mechanism of communication exists in the SCN. Future in vitro electrophysiological experiments should provide an explanation of how neurotransmitters, local neuronal circuits and intrinsic membrane properties regulate the electrical activity of SCN neurons during the circadian rhythm.

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ELECTROPHYSIOLOGY OF THE SUPRACHIASMATIC NUCLEUS: SYNAPTIC TRANSMISSION, MEMBRANE PROPERTIES, AND NEURONAL SYNCHRONIZATION

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ABSTRACT

Knowledge of the neuronal membrane properties and synaptic physiology of the suprachiasmatic nucleus (SCN) is critical for an understanding of the cellular basis of circadian rhythms in mammals. The hypothalamic slice preparation from rodents and a combination of electrophysiological techniques (i.e., extracellular single- and multiple-unit recording, intracellular recording, and whole-cell patch clamp) were used to study (1) the role of excitatory and inhibitory amino acids (i.e., glutamate and GABA) in synaptic transmission, (2) the membrane properties of SCN neurons, and (3) the mechanisms of neuronal synchronization. Antagonists for N-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors blocked excitatory postsynaptic potentials (EPSPs) evoked by stimulation of the optic nerve or other sites when SCN cells were depolarized or at rest, respectively. Bicuculline blocked inhibitory postsynaptic potentials (IPSPs) that were evoked by local stimulation or that occurred spontaneously. The IPSP reversal potential was near the Cl^- equilibrium potential, and was shifted to depolarized levels by raising intracellular $[Cl^-]$. Thus, glutamate and GABA appear to mediate fast excitatory and inhibitory synaptic transmission in the SCN. Some SCN neurons, but not all of them, had low-threshold Ca^{2+} spikes and time-dependent inward rectification, thus indicating that the electrical properties of SCN neurons are not homogenous. Neurons with a firing rate >6 Hz had a regular pattern, and neurons with a rate <4 Hz had an irregular pattern; since both the firing rate and pattern could be modified with injected currents, SCN neurons with different firing patterns are unlikely to represent distinct classes of cells. Synchronous bursts of action potentials occurred in the SCN after chemical synapses were blocked with $[Ca^{2+}]$-free solutions and with amino-acid-transmitter antagonists, which indicates that synchronous neuronal activity can occur in the SCN without active chemical synapses and suggests

that a different mechanism of communication exists in the SCN. Future in vitro electrophysiological experiments should provide an explanation of how neurotransmitters, local neuronal circuits and intrinsic membrane properties regulate the electrical activity of SCN neurons during the circadian rhythm.

Introduction

Although considerable information is available concerning the important role of the SCN in the generation of circadian rhythms (Klein et al., 1991), very little is known about the electrophysiological properties of SCN neurons at the cellular and membrane level. The relatively small size ($\sim 10\ \mu m$) of SCN neurons had made it difficult to record intracellularly with traditional sharp-electrode techniques, even in slice preparations. The few available studies that used intracellular recordings in hypothalamic slices (Wheal and Thomson, 1984: Sugimori et al., 1986; Thomson and West, 1990) did provide fundamental information on the physiological properties of SCN neurons, but they left many unanswered questions about the transmitters responsible for EPSPs and IPSPs, the homogeneity of the membrane properties, and the cellular mechanisms of synchronization of electrical activity. The experiments described below were aimed at (1) identifying the receptors mediating EPSPs and IPSPs, (2) identifying the intrinsic electrical properties of SCN neurons, with an emphasis on the presence of low-threshold Ca^{2+} spikes and time-dependent inward rectification and on the issue of the homogeneity/heterogeneity of SCN electrical properties, and (3) determining whether Ca^{2+}-dependent chemical synaptic transmission is required for the synchronization of electrical activity in the SCN.

Excitatory amino acids

The role of excitatory amino acid receptors in the generation of excitatory synaptic responses to optic nerve stimulation was studied in horizontal and parasagittal hypothalamic slices from rats and guinea pigs (Kim and Dudek, 1991). Activation of retinal input evoked EPSPs in SCN neurons.

The antagonist for AMPA/kainate-type receptors, 6,7-dinitroquinoxaline-2,3-dione (DNQX, 1-10 μM), blocked EPSPs in a concentration-dependent and reversible manner. The selective NMDA-receptor antagonist, DL-2-amino-5-phosphonopentanoic acid (AP5, 50-100 μM) did not significantly and consistently affect the EPSPs at resting or hyperpolarized membrane potentials; however, when SCN neurons were depolarized, AP5 blocked or depressed a slow component of the EPSPs. Similar data were obtained for spontaneous EPSPs and EPSPs to stimulation at other sites. These results demonstrate that both non-NMDA and NMDA receptors mediate excitatory synaptic transmission both from retinal input and from other CNS sites, and that NMDA receptors contribute to EPSPs in depolarized SCN neurons, in agreement with the removal of Mg^{2+} block during depolarization.

Gamma-amino-butyric acid (GABA)

The mechanisms of inhibitory synaptic transmission were also studied (Kim and Dudek, 1992). Electrical stimulation dorsocaudal to the SCN evoked fast ISPSs in most neurons, and spontaneous IPSPs were present in every neuron. Spontaneous and evoked IPSPs were hyperpolarizing at resting potential and had a reversal potential $\sim$-75 mV. With KCl electrodes, the IPSPs were positive-going. The IPSPs were blocked by bicuculline, a $GABA_A$ receptor antagonist. Bicuculline-resistant hyperpolarizing potentials, similar in time-course to the fast IPSPs, occurred spontaneously in some cells and could be evoked by electrical stimulation of the optic nerve or a dorsocaudal site. A fast prepotential (i.e., a partial action potential) always preceded these hyperpolarizing potentials, and injection of hyperpolarizing currents blocked these events, thus indicating they were not synaptic in origin. The properties of these hyperpolarizations indicate that they represent the passive reflection of the hyperpolarizing afterpotentials from blocked action

potentials (e.g., dendritic spikes or electrotonic coupling potentials). No slow IPSPs were detected in SCN neurons. Therefore, SCN neurons receive extensive GABAergic input; $GABA_A$ receptors and an increase in Cl^- conductance mediate these IPSPs.

Membrane properties

Electrophysiological properties were studied to determine if SCN neurons are homogeneous or heterogeneous, and if distinct classes of neurons could be identified (Kim and Dudek, 1993). The experiments focused on the subpopulation of neurons that demonstrably received retinal input, as determined by recording short-latency EPSPs to optic nerve stimulation (Kim and Dudek, 1991). Individual action potentials were relatively short in duration, and followed by a pronounced hyperpolarizing afterpotential. Spike inactivation, spike broadening and frequency accommodation occurred consistently during depolarizing current pulses, and an after-hyperpolarization routinely followed a burst of action potentials. With sharp electrodes, the membrane time constant of these neurons ranged from 7 to 21 msec (mean 11.4 $\pm$ 0.7 msec), and input resistance was 105 to 626 MΩ (mean 301 $\pm$ 23 MΩ). Although there was some variability in electrical properties, no distinct groups were found when analyses were made across the neuronal population. Some neurons did show slight time- and voltage-dependent inward rectification, and these neurons had a higher spontaneous firing rate and were more excitable. Some neurons also had low-threshold Ca^{2+} spikes, although other neurons clearly lacked them. Most neurons fired spontaneously; those neurons with a firing rate >6 Hz had a regular firing pattern, whereas neurons that fired <4 Hz had an irregular pattern, as reported previously by Thomson et al. (1984). Altering the firing rate with injected current changed the firing pattern. These results suggest that: (1) SCN neurons receiving optic

nerve input are not electrophysiologically homogeneous, but they do not yet appear to form distinct classes of electrophysiological cell types, (2) time-dependent inward rectification and the capacity to generate low-threshold Ca^{2+} spikes are limited to only a subpopulation of SCN neurons, (3) time-dependent inward rectification is associated with an increased spontaneous firing rate and excitability, and (4) firing pattern is related to firing rate, probably more than to cell type.

Non-chemical-synaptic mechanisms of synchronization of SCN neurons

Several independent observations concerning the SCN and circadian rhythms have suggested that SCN neurons can be synchronized by mechanisms that do not involve Na^{+}-mediated action potentials and chemical synaptic transmission (e.g., Schwartz et al., 1987). When hypothalamic slices were bathed in a $[Ca^{2+}]$-free solution for several hours, bursts of action potentials occurred in the SCN (Bouskila and Dudek, 1993). Multiple-unit recordings showed that populations of SCN neurons had their bursts of activity roughly synchronized, and dual recordings from adjacent areas confirmed that in one SCN the bursts occurred synchronously across the population. The bursts in one SCN were not, however, synchronized with bursts in the contralateral SCN. A mixture of NMDA, non-NMDA, and $GABA_A$ receptor antagonists had no effect upon the synchronicity of the bursts. Whole-cell patch-clamp recordings confirmed that the $[Ca^{2+}]$-free solution blocked the evoked EPSPs and IPSPs, and the mixture of antagonists blocked the remaining spontaneous PSPs. These results indicate that synchronous neuronal activity can occur in the SCN without active chemical synapses, thus strongly suggesting that a different mechanism of communication exists in the SCN.

Discussion and Conclusions

Previous extracellular studies provided evidence that retinal input via the optic nerve had an excitatory effect on SCN neurons, and that glutamate acting on non-NMDA receptors was the probable transmitter (Cahill and Menaker, 1989a,b). The preliminary studies of Thomson and West (1990) suggested that GABA was the transmitter responsible for spontaneous IPSPs in SCN neurons. We found that retinal input generated fast EPSPs, but no IPSPs; stimulation of other sites around the SCN caused both fast EPSPs and fast IPSPs (Kim and Dudek, 1991, 1992). Glutamate, acting on non-NMDA receptors at resting potential and NMDA receptors when the neurons are depolarized, appears to mediate most if not all fast EPSPs (Kim and Dudek, 1991). Only fast IPSPs (no slow IPSPs) were observed in SCN neurons; $GABA_A$ receptors and an increase in Cl^- conductance appeared to generate these IPSPs (Kim and Dudek, 1992). The pharmacological and ionic properties of fast synaptic events appear to be identical to other hypothalamic neurons, including those in the supraoptic, paraventricular, and arcuate nuclei (van den Pol et al., 1990; Wuarin and Dudek, in press).

Although intrinsic electrophysiological properties were described in previous intracellular studies of the SCN (see Introduction), little or no data are available on whether the electrical properties are the same across all the neurons in the SCN. In our experiments, the electrophysiological properties of SCN neurons did not appear to be homogeneous, since some neurons had low-threshold Ca^{2+} spikes and time-dependent inward rectification and yet others did not (Kim and Dudek, 1993). One classification scheme, based on the electrical activity of SCN neurons, has relied on the type of firing pattern (i.e., regular versus irregular). Our experiments, however, suggest that firing pattern is a function of membrane potential and firing rate (see Thomson

et al., 1984). Therefore, although the electrical properties of SCN neurons do not appear homogeneous, distinct classes of SCN neurons based on electrophysiological characteristics are not yet available. Future whole-cell patch-clamp studies may yet identify specific classes of SCN neurons.

The conclusion that the SCN displays a circadian rhythm of electrical activity is derived from studies using populations of single-unit recordings (e.g., Gillette, 1991); therefore, the circadian rhythm of electrical activity appears to be a property of a population of SCN neurons, which strongly suggests that a mechanism exists to synchronize at least some of the SCN neurons involved in circadian time keeping. Our experiments showed that synchronization of neuronal activity into bursts of action potentials could occur in $[Ca^{2+}]$-free solutions that block chemical synaptic transmission (Bouskila and Dudek, 1993). Therefore, although excitatory and inhibitory amino acids appear to mediate all fast postsynaptic potentials in the SCN, Ca^{2+}-dependent chemical synaptic transmission is not necessary for neuronal synchronization. The possible mechanisms include electrotonic coupling via gap junctions among SCN neurons, ephaptic interactions, and shifts in the concentration of extracellular ions such as K^+. Future experiments will attempt to evaluate these mechanisms in the SCN.

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